

18. (Previously Amended) A kit of parts comprising means for performing the method according to claim 1.

19. (Previously Amended) The kit of parts according to claim 18, wherein said kit of parts is designed for mass-screening purposes.

IN THE SPECIFICATION:

A clean version of each paragraph of the as-filed specification amended herein is provided below, for clarity. A version of each of the amended paragraphs with markings to show changes made relative to the as-filed version of such amended paragraphs is enclosed herewith.

Please replace the paragraph located at **page 2, line 24, through page 3, line 7**, of the as-filed specification with the following replacement paragraph:

Thus far, the only known component of the infectious prion is an abnormal, disease-causing isoform of the "normal" prion protein (PrP) called PrP^{Sc} or aberrant prion protein. PrP, or normal prion protein, is ubiquitous in mammalian cells in a benign, cellular conformation (PrP^C) and is encoded within a single exon as a protein of about 250 amino acid residues (FIG. 1) (SEQ ID NOS:1-6). The PrP gene has been cloned and sequenced from a variety of species, and there is a high degree of structural and organizational homology between mammalian PrP sequences (Schatzl et al., 1995). PrPs in many mammals have a 22-24 residue long N-terminal signal sequence as well as a 22-24 residue long C-terminal signal sequence for attachment of a GPI-anchor. This glycosylphosphatidylinositol linkage is a fairly common means of anchoring proteins to membranes of eukaryotic cells. Further structural characteristics of the mature protein (of 206-210 amino acid residues) are one disulfide bond and two sites for Asn-linked glycosylation.

Please replace the paragraph located at **page 3, lines 8-23**, of the as-filed specification with the following replacement paragraph:

PrP^{Sc} originates from the normal cellular isoform (PrP^C) by a post-translational process since the amino acid sequence of PrP^{Sc} is identical to that predicted from cDNA or genomic nucleic acid sequences. Glycosylation patterns are also identical between PrP^C and PrP^{Sc}. Moreover, Caughey & Raymond (1991) demonstrated that PrP^{Sc} is made from a cell surface precursor that is identical to the normal PrP. PrP^{Sc} differs from the normal, membrane-bound cellular prion protein by its relative protease resistance. Treatment with proteinase K (PK), for instance, results in complete proteolysis of PrP^C, whereas in PrP^{Sc}, the N-terminal part is removed before the amino acid at position 90 (human numeration) (SEQ ID NO:1). The protease-resistant core left is designated PrP27-30 after its electrophoretic behavior in SDS-PAGE as a protein molecule with M_r = 27-30 kDa, and this molecular species retains full infectivity.

Please replace the paragraph located at **page 12, lines 4-24**, of the as-filed specification with the following replacement paragraph:

Capillary electrophoresis was adapted by Schmerr et al. (1995, 1996, 1998a) as a diagnostic, immunochemical assay for scrapie. The authors claim a high sensitivity (approx. 135 pg PrP^{Sc}) of their test by measuring laser-induced fluorescence of a PrP-derived fluorescein-labeled peptide after its separation by free zone capillary electrophoresis. In a preceding competition step, this peptide was displaced from a preformed complex of the peptide and an antibody directed to the unlabeled peptide in competition with the analyte (PrP^{Sc}). Beforehand, PrP^C had been removed from the analyte solution by PK-treatment. The concentration of rabbit antiserum for complex-preformation was chosen so that the antibody would be limiting in the assay (adjustment to 50% of the maximum amount of immunocomplex). Four anti-(prion)-peptide antisera were prepared and evaluated. Assays using antisera to the peptides spanning mouse amino acid position 142-154 (SEQ ID NO:4) and 155-178 (SEQ ID NO:4) differentiated scrapie-positive sheep from normal animals. In spite of the high sensitivity of this method, sample processing is time-consuming (approx. 24h) and

cumbersome since PrP^{Sc} from brain stem has to be concentrated and purified through steps like ultracentrifugation and HPLC.

Please replace the paragraph located at **page 13, lines 7-16**, of the as-filed specification with the following replacement paragraph:

Race et al. (1992) could find PrP^{Sc} in every brain of 8 sheep that were histologically positive for scrapie and even in brains of clinically positive sheep that were not diagnosed as scrapie-positive by histology. For detection, anti-peptide antibodies to residues 89-103 (SEQ ID NO:4) and 218-232 (SEQ ID NO:4) of the mouse PrP sequence were used. Apparently, the amount of tissue required to visualize PrP^{Sc} varied among sheep from <2 to 200 mg equivalents of brain tissue. Also, PrP^{Sc} was found in spleens and lymph nodes in 7 of 8 sheep that had the protease-resistant form detected in brain homogenates.

Please replace the paragraph located at **page 13, line 26, through page 14, line 10**, of the as-filed specification with the following replacement paragraph:

Thus far, two commercial assays have been announced. In 1997, the Swiss company Prionics Inc. launched its "BSE Western Test" intended for mass screening of slaughter cattle. A modified and optimized Western blot method was used to detect the proteinase K-resistant PrP²⁷⁻³⁰ in bovine brain stem. For immunodetection mAb 6H4 was used, developed by immunizing PrP-null mice with recombinant bovine PrP. This antibody recognizes residues 147-155 (SEQ ID NO:5) of the bovine sequence as a linear epitope in native PrP^C and denatured PrP^{Sc}; this sequence is also recognized in sheep, human, pig and mouse. Incubation with anti-mouse IgG coupled to alkaline phosphatase and detection of the enzymatic product by chemiluminescence were the final steps of the assay. This test requires an incubation step with PK and detects PrP²⁷⁻³⁰. Reliability is strengthened by the Western blot documentation of the decrease in size (internal control) of the prion protein from 30-33 to 27-30 kDa. The test can be done within hours, and the expectation is that subclinical BSE in post-mortem brains may be detected.

Please replace the paragraph located at **page 19, line 29, through page 20, line 9**, of the as-filed specification with the following replacement paragraph:

The invention also provides a method comprising immunological detection of said aberrant prion protein using at least one antibody directed against the aberrant protein, preferably directed against a proteinase K-resistant part of the aberrant prion protein, for example wherein said antibody is directed against a proteinase K-resistant N-terminal part of the aberrant prion protein. Monoclonal or polyclonal antibodies can be used, possibly said antibody is raised against a peptide derived from the prion protein, for example wherein said peptide is selected from an N-terminal group consisting of residues 94-111 (like 94-105 (SEQ ID NOS:7-11) and 100-111 (SEQ ID NOS:12-15), a C-terminal group consisting of residues 223-234 (SEQ ID NOS:27-30) and a group consisting of residues 145-177 (SEQ ID NOS:21-26) (sheep numbering) or sequential homologues of the PK-resistant part of PrP^{Sc} (FIG. 2) or functional equivalents thereof.

Please replace the paragraph located at **page 22, line 20, through page 23, line 14**, of the as-filed specification with the following replacement paragraph:

Primary antibodies

These were intentionally designed for scrapie diagnosis. Antisera were induced in rabbits using synthetic peptides with sequences based on the sequence of ovine PrP protein. The sequences have such differences with the rabbit PrP sequence that they induce not only antibodies which recognize these peptides but also the authentic PrP protein. Other animal species like mouse, which have sequence differences could be suitable as well. The sequences used for immunization were selected from the protease K-resistant domain of PrP^{Sc}. The selected 12-mer sequences (SEQ ID NOS:11, 12, 30) represent domains that have a low tendency to form secondary structure (α -helix or β -sheet). The antisera are reactive in diagnostic dot blotting but also in Western blotting of both PrP^C and PrP^{Sc}, in ELISAs with, as coated antigens, the above peptides or PrP protein, and in immunohistochemical detection. With the peptide derived from the ovine prion protein sequence 94-105 (SEQ ID NO:11), antisera R521 and R522 were produced in rabbits. Likewise, sequence 100-

111 (SEQ ID NO:12) yielded antisera R504, R505, R593, R594, R595, and R596, and sequence 145-177 (SEQ ID NO:26) antiserum R532. The sequence 126-143 (ovine and bovine) (SEQ ID NO: 20) gave rise to antiserum R568, while sequence 223-234 (ovine and bovine) (SEQ ID NO:30) yielded antisera R523 and R524. Peptides were synthesized and used to raise anti-peptide antisera in rabbits following previously published procedures (Van Keulen et al., 1995). Antisera were confirmed to be specific for sheep PrP (both undigested and after proteinase K treatment) on Western blots of partially purified prion protein from scrapie-affected sheep brain.

Please replace the paragraph located at **page 26, lines 26-34**, of the as-filed specification with the following replacement paragraph:

Five classes of anti-peptide antisera to linear epitopes of sheep PrP sequences (94-105 (SEQ ID NO:11), 100-111 (SEQ ID NO:12), 126-143 (SEQ ID NO:20), 145-177 (SEQ ID NO:26), and 223-234 (SEQ ID NO:30)) were examined. For comparative reasons, all sera were used in a 1/500 dilution in PBTS. Antisera to the 94-105 sequence (SEQ ID NO:11) (R521, R522) and to the 100-111 sequence (SEQ ID NO:12) (R505) proved to have the best differentiating power. On the other hand, with the antisera R568 and R532 to the sequences 126-143 (SEQ ID NO:20) and 145-177 (SEQ ID NO:26), respectively, no immunoenhancing effect of 4 M gdnSCN on PrP^{Sc} could be detected.

Please replace the paragraph located at **page 27, lines 12-26**, of the as-filed specification with the following replacement paragraph:

From brains of BSE-positive cattle, obtained from The Netherlands, the UK, Ireland, Belgium and Switzerland and of Dutch BSE-negative cattle (diagnosed by histopathology and immunohistochemical examination), brain stems were extracted with lysis buffer in the same manner as for sheep, and the low-speed supernatant 1 was used for further examination. Brain stem extracts from confirmed scrapie-negative and positive sheep were used for comparison. Aliquots of extracts were also treated with proteinase K and 3 µl amounts of dilutions in lysis buffer of PK-treated and

untreated extracts were spotted onto NC membranes. Immunodetection was with 1/1000 dilutions of antisera to the 12-mer sequences 94-105 (SEQ ID NO:11) (antiserum R521), 100-111 (SEQ ID NO:12) (R505, R595, R596), 223-234 (SEQ ID NO:30) (R523, R524) and to the longer sequences 126-143 (SEQ ID NO:20) (R568) and 145-177 (SEQ ID NO:26) (R532).

Please replace the entire contents of **pages 36 and 37** of the as-filed specification with the following:

LEGENDS TO THE FIGURES

FIG. 1:

Amino acid sequences of human (SEQ ID NO:1), rabbit (SEQ ID NO:2), hamster (SEQ ID NO:3), mouse (SEQ ID NO:4), cattle (SEQ ID NO:5) and sheep (SEQ ID NO:6) PrP genes. The entire amino acid sequence of human PrP is given (SEQ ID NO:1); open spaces in the other sequences indicate identity (SEQ ID NOS:2-6). Polymorphisms are indicated in bold at the top of each block and relate to the shaded positions. ↓: PHGGGWGQ. †: protease-sensitive site, right of which the sequence for the PK-resistant core of PrP^{Sc} is found. The mature PrP is devoid of N and C terminal signal peptides (in *huPrP* (SEQ ID NO:1): amino acids 1-22 and 232-253, respectively).

human (SEQ ID NO:1):

- Kretzschmar, H.A., Prusiner, S.B., Stowring, L.E. & DeArmond, S.J. (1986), "Scrapie prion proteins are synthesized in neurons," *Am. J. Pathol.* 122:1-5.

rabbit (SEQ ID NO:2):

- Loftus, B. & Rogers, M. (1997), "Characterization of a prion protein (PrP) gene from rabbit: a species with apparent resistance to infection by prions," *Gene* 184:215-219.
- Rubenstein, R., Kasesak, R.J., Papini, M., Kasesak, R., Carp, R.I., Lafauci, G., Meloen, R., & Langeveld, J. (1998) *J. Neuroimmunology* (accepted).

golden Syrian hamster (SEQ ID NO:3):

- Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M., Groth, D.F., McKinley, M.P., Prusiner, S.B., & Weissman, C. (1986), "Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene," *Cell* 46:417-428.

mouse (SEQ ID NO:4):

- Loch, C., Chesebro, B., Race, R. & Keith, J.M. (1986), "Molecular cloning and complete sequence of prion protein cDNA from mouse brain infected with the scrapie agent," *Proc. Nat'l Acad. Sci. USA* 83:6372-6376.
- Westaway, D., Goodman, P.A., Mirenda, C.A., McKinly, M.P., Carlson, G.A. & Prusiner, S.B. (1987), "Distinct prion proteins in short and long scrapie incubation period mice," *Cell* 51:651-662.

cattle (SEQ ID NO:5):

- Goldmann, W., Hunter, N., Martin, T., Dawson, M. & Hope, J. (1991), "Different forms of the bovine PrP gene have five or six copies of a short, g-c-rich element within the protein-coding exon," *J. Gen. Virol.* 72:201-204.

sheep (SEQ ID NO:6):

- Goldmann, W., Hunter, N., Foster, J.D., Salbaum, J.M., Beyreuther, K. & Hope, J. (1990), "Two alleles of a neural protein gene linked to scrapie in sheep," *Proc. Nat'l Acad. Sci. USA* 87:2476-2480.

FIG. 2:

Peptide sequences derived from the prion protein structures of six species (*hu*=human (SEQ ID NOS:7, 12, 16, 21, 27), *rb*=rabbit (SEQ ID NOS:8, 13, 17, 22, 28), *ha*=hamster (SEQ ID NOS:9, 14, 18, 23, 29), *mo*=mouse (SEQ ID NOS:9, 14, 19, 24, 29), *bo*=cattle (SEQ ID NOS:10, 15, 20, 25, 30), *ov*=sheep (SEQ ID NOS:11, 12, 20, 26, 30)). The amino acid sequence of the human peptides is given (SEQ ID NOS:7, 12, 16, 21, 27); open spaces in the other sequences indicate identity (SEQ ID NOS:8-11, 13-15, 17-20, 22-26, 28-30). Antipeptide antibodies were raised in rabbits against the peptides of the ovine structure. Corresponding antisera are indicated **R5xx** at the top of each set of sequences. The set of sequences under the heading **R521, R522 (to ovine sequence 94-105)** includes the amino acid sequences of *hu*=human (SEQ ID NO:7), *rb*=rabbit (SEQ ID NO:8), *ha*=hamster (SEQ ID NO:9), *mo*=mouse (identical to hamster) (SEQ ID NO:9), *bo*=cattle (SEQ ID NO:10), and *ov*=sheep (SEQ ID NO:11). The set of sequences under the heading **R504, R505, R593-596 (100-111)** includes the amino acid sequences of *hu*=human (SEQ ID NO:12), *rb*=rabbit (SEQ ID NO:13), *ha*=hamster (SEQ ID NO:14), *mo*=mouse (identical to hamster) (SEQ ID NO:14), *bo*=cattle (SEQ ID NO:15), and *ov*=sheep (identical to human) (SEQ ID NO:12). The set of sequences under the heading **R568 (126-143)** includes the amino acid sequences of *hu*=human (SEQ ID NO:16), *rb*=rabbit (SEQ ID NO:17), *ha*=hamster (SEQ ID NO:18), *mo*=mouse (SEQ ID NO:19), *bo*=cattle (SEQ ID NO:20), and *ov*=sheep (identical to cattle) (SEQ ID NO:20). The set of sequences under the heading **R532 (145-177)** includes the amino acid sequences of *hu*=human (SEQ ID NO:21), *rb*=rabbit (SEQ ID NO:22), *ha*=hamster (SEQ ID NO:23), *mo*=mouse (SEQ ID NO:24), *bo*=cattle (SEQ ID NO:25), and *ov*=sheep (SEQ ID NO:26). The set of sequences under the heading **R523, R524 (223-234)** includes the amino acid sequences of *hu*=human (SEQ ID NO:27), *rb*=rabbit (SEQ ID NO:28), *ha*=hamster (SEQ ID NO:29), *mo*=mouse (identical to hamster) (SEQ ID NO:29), *bo*=cattle (SEQ ID NO:30), and *ov*=sheep (identical to cattle) (SEQ ID NO:30).